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Morphometric forms, biovolume and cellular carbon content of dinoflagellates from polluted waters on the Karachi coast, Pakistan

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Present study reports new information on the biovolume and carbon biomass estimates for dinoflagellates from Manora Channel, Karachi coast, Pakistan. Biovolume per cell was calculated using the geometric shape of dinoflagellates at species level. Both thecate and athecate species were examined under light and scanning electron microscope. A total of 45 species were measured and their cell size was ranged between 20-450µm. Geometric forms of the species were seven classed into as ellipsoidal, spherical, double cone shape, prolate sphere, cone and half sphere, "cone+3 cylinder" shape, "ellipsoidal + 2 cone+ cylinder" shape, "cylinder+ cone" shaped. Total biovolume ranged from 3.743×10^3 to 2.2×10^5 µm^3 cell^{-1} and estimated cellular carbon content per cell ranged from 397×10^2 to 26.5×10^4 pg C cell^{-1}. Carbon and biovolume relationship was significant for thecate species which can thus be used for carbon flux studies.

[**Keywords:** *Biovolume*, Carbon biomass, Dinoflagellates, Pakistan]

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Introduction

Dinoflagellates ranging from (5–2000 µm in diameter) are a significant proportion of total phytoplankton biomass in oceanic ecosystems[1]. Some of these aquatic microorganism are toxic and harmful, and can then negatively impact water quality and cause public health risks as well as economic loss to fisheries resources[2,3]. Dinoflagellates also contribute to carbon flux and thereby provide energy to deep-water and demersal fish[3,4]. To determine the ecology as fully as possible, abundance, cell biovolume, cell biomass, species succession, growth and cell loss processes should be estimated[4,5,6,7]. Sizes and shapes plays important in numerical censuses of abundance, grazing, cellular processes and other metabolic process. Coastal physical forcing (wind, circulation, horizontal transport and eddy turbulence) affects cell density and surface area to volume ratio[4]. Surface areas to volume ratio have been used to estimate the contribution of small cells, which generally contribute a significant fraction of the total biomass, rather than large cells alone[8]. Small species have a reduced boundary layer and larger surface area per unit volume, which allows them to acquire relatively more nutrients [9].

Carbon fluxes estimation requires data on cell biovolume[5,6]. Several techniques have been employed to calculate phytoplankton biovolume, such as optical and image analysis software[10], electronic particle counting[11], flow cytometry[12,13], and computer tomography such as holographic Scanning techniques[14]. Cell biovolume and cellular carbon content can be estimated from chlorophyll *a*, ATP, cell-nitrogen concentration and depth variation[15, 16,17], as well as from geometric shape measurement [5,6, 18,19].

Geometric shape models and biovolume estimates for dinoflagellates and other phytoplankters have been made for Chinese Sea[6, 20], the Baltic Sea[21], the Mediterranean Sea[7] and India[22]. Biovolume and carbon biomass for dinoflagellates reported in this paper provide original baseline data for the Pakistan coast, northern Arabian Sea.

Materials and Methods

Phytoplankton samples were collected from two sites in the Manora Channel, off the coast from Karachi, Pakistan (Fig. 1). Bimonthly samples (n=180) were collected using a 1.7 L Niskin bottle from May 2002 to July 2003, and fixed with Lugol's preservative. Samples were examined by light microscope using the Utermöhl method[23] and species identifications were confirmed by scanning and fluorescence microscopy.

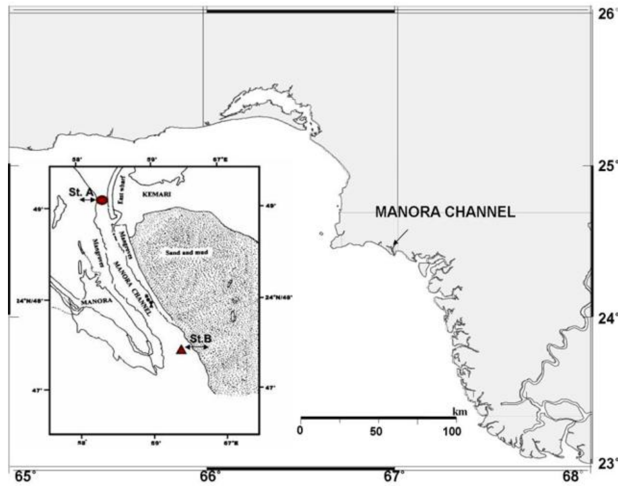


Fig. 1. Map of Karachi coast, Pakistan. Locations indicated by circle (red dot) for St. A and triangle (red triangle) for St. B.

Species' biovolumes (as μm^3 $cell^{-1}$) were calculated from assigned geometric shape Sun and Liu 2003, and at least 30 to 300 cells of each species were measured[4] . The cellular carbon content (pg C $cell^{-1}$) was calculated from the formula of Eppley (1970):

$$\text{Log pg C cell}^{-1} = \text{Power } 10\ 0.94 \times \text{Log V}(\mu m^3) - 0.60$$

Results

Community structure and morphometric shapes

At both stations, a total of 45 species of 20-450 µm were divided into sizes classes. Seven geometric forms, determined from the measurements under light/scanning electron microscopy, were used to calculate biovolume.

Ellipsoidal-shaped species were *Akashiwo sanguinea*; *Alexandrium ostenfeldii*;, *A. tamarense*; *A. tamiyavanichi*; *Dinophysis caudata*; *D. acuminata*; *Gymnodinium* sp.; *Gyrodinium spirale*; *Gyrodinium* sp.; *Prorocentrum arcuatum*; *P. micans*; *P. gracile*; *P. donghaiense*; *P. minimum*; *Phyrophacus steinii* (Plate I, Fig 2). Double-cone-shaped species were *Ceratium fusus*; *C. inflatum*; *Gonyaulax spinifera*; *Protoperidinium steinii*; *P. divergens*; *P. depressum* (Plate II, Fig 3), a cone-and-half-sphere shaped species was *Scrippsiella trochoidea*, and spherical species were *Protoceratium reticulatum*; *P. balticum* (Plate III, Fig 4) and complex-shaped species were 2 cones + 3 cylinders *C. furca*, *C. lineatum*; ellipsoidal + 2 cones + 1 cylinder was *C. macroceros*, (Plate IV, Fig 5), while a prolate sphere shaped species (*Cochlodinium* cf. *fulvescens*).

Biovolume and carbon-biomass

Biovolumes and carbon content for all species are listed in Table 1. Biovolumes of species ranged between 2535 to 220893 μm^3 $cell^{-1}$ and carbon content ranged from 397 to 33016 pg C $cell^{-1}$. A total of 31 autotrophic/mixotrophic and 14 heterotrophic species had cell densities ranging from 20 cells/L to 48,166 cells/L (Table 1). Biovolume and carbon content of the autotrophic/mixotrophic species ranged from 4088 to 105500 μm^3 and 623 to 16684 pg C $cells^{-1}$, respectively, at station A and 2535 to 147262 μm^3 and 397 to 18114 pg C $cells^{-1}$, respectively, at station B (Table 1). Biovolume and carbon content of heterotrophic species at Station A had values ranging from 3151 to 220893μm^3 and 571 to 33916 pg C $cells^{-1}$, respectively, and 5473 to 149500 μm^3 and 810 to 23316 pg C $cells^{-1}$, respectively, at station B (Table 1). Lowest carbon per cell was observed for *P. minimum*/*P. balticum* at station B and highest carbon per cells was observed for *P. depressum* at station A.

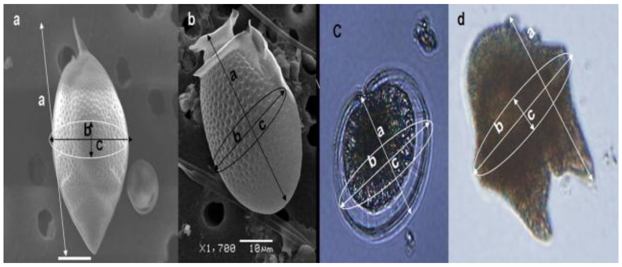


Fig. 2, Plate 1. LM and SEM's of ellipsoidal shape *Prorocentrum micans* (A), *Dinophysis acuminata* (B), *Pyrophacus steinii* (C), *Akashiwo sanghainea* (D) represented by length (a), breath (b), depth (c) and biovolume V: 3.14/6. a.b.c (Sun and Liu 2003).

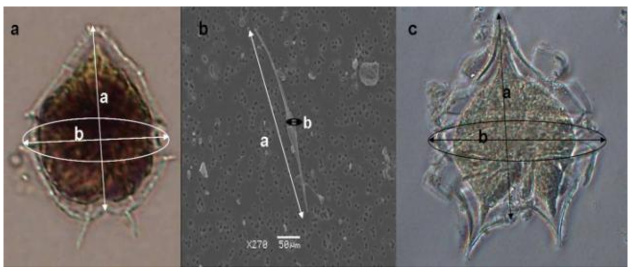


Fig. 3, Plate II. Light and scanning electron microscopy, double cone shape species, *Gonayaulax spinifera* (A), *Ceraitum fusus* (B), *Protoperidinium divergens* (C) represented by length (a), width (b), depth (c). Formula computed by biovolume V: pi/12. $a.b^2$. Scale bar= 10 µm, 50 µm

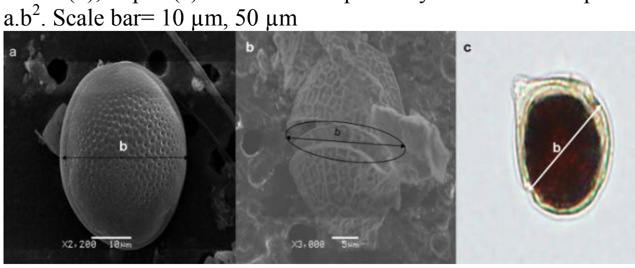
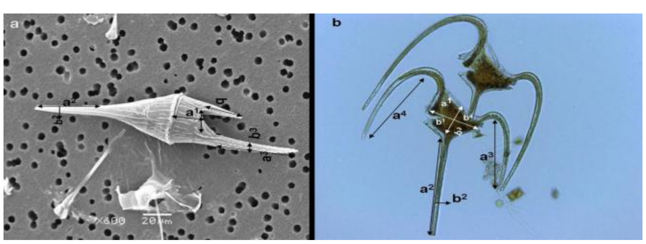


Fig. 4, Plate III. SEMs of sphere shaped *Prorocentrum compressum* (A), *Protoceraitum reticulatum* (B) represented by geometric calculation diameter (b) and biovolume computed by V: $pi/6.a^3$ and , cone and half shape *Scrippsiella trochoidea* (C) and biovolume computed by V: $3.14/4.a.b^2$



Fig, 5, Plate IV. Lm and SEMs of complex shape species *Ceraitum furca* (Cone+3 cylinder) by geometric calculation by formulaV:$3.14/4.a_2.b_2^2+3.14/2.a_3.b_3^2+3.14/12.a_1.(b_1^2+b_1.b_2+b_2^2)$ and *C. macroceros* (Ellipsoid + 2 cones+ cylinder) represented by V: $3.14/4.a_2.b_2^2$ +3.14/12 (a3+a4).

Biovolume and carbon biomass calculated on single cell basis of all species studied had significant relationship (linear coefficient relationship). A total of 33 thecates and 4 athecates species showed significant correlation with carbon biomass (Figures 6 & 7), although thecates species appeared to have slightly higher calculated carbon content at Stn A (R^2 = 0.993, *P*> 0.0001) and Stn B (R^2 = 0.978, *P*> 0.0001) than athecate species (R^2 = 0.82, Stn A) and (R^2 = 0.99, *P*> 0.01, Stn B). It is interesting to note that smaller cells contribute more carbon biomass per liter of water compared to large cells.

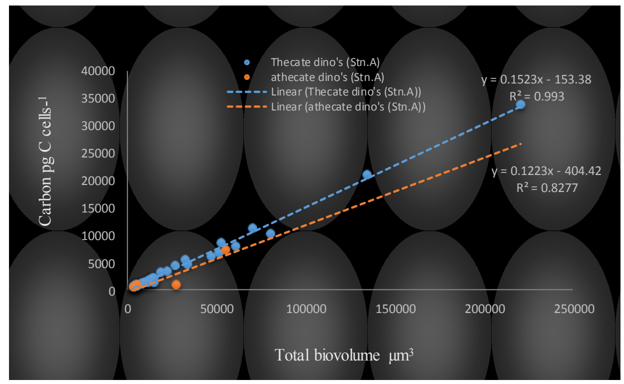


Fig. 6, Linear regression between biovolume and carbon per cells of thecate and athecate dino's at Station A.

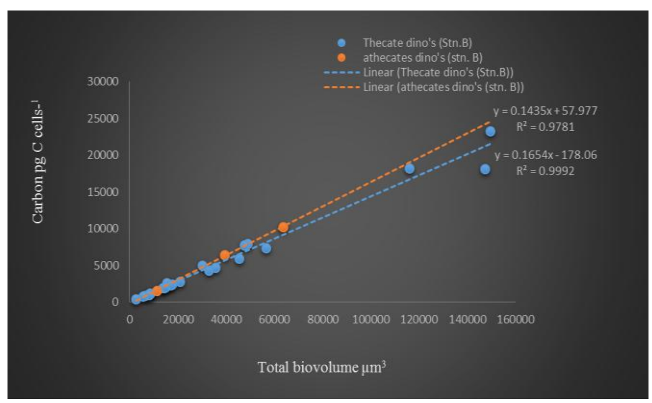


Fig. 7, Linear regression between biovolume and carbon per cells of thecate and athecate dino's at Station B.

Table 1. The biovolume and carbon content estimation for dinoflagellates from Karachi waters.

Autotrophic/ Mixotrophic	Station A			Station B		
	Max cells/L	Biovolume μm^3	Carbon Pg C $cells^{-1}$	Max cells/L	Biovolume μm^3	Carbon Pg C $cells^{-1}$
Akashiwo sanguinea	40	27750	1000	-	-	-
Alexandrium ostenfeldii	1440	8181	1197	2120	14137	2001
A. tamarense	520	5236	787	-	-	-
A. tamiyavanichi	225	14392	2035	-	-	-
Ceratium furca	75	12371	1765	640	20697	2864
C. fusus	80	51516	6749	906	32598	4389
C. inflantum	80	46952	6185	106	45363	5988
C. kofoid	-	-	-	40	15332	2619
C. lineatum	106	14729	2080	80	17338	2425
C. macroceros var macroceros	-	-	-	160	6313	938
C. massilliense	-	-	-	40	30050	4997
C. pelluchellum	-	-	-	40	48782	7956
C. longipes	-	-	-	40	47476	7752
Cochlodinium sp	160	5724	856	926	39208	6451
Dinophysis caudata	94	80799	10303	392	35540	4761
D. acuminata	80	34088	4634	-	-	-
D. miles	80	15228	1382	-	-	-
D. fortii	40	52675	8565	-	-	-
D. infundibula	20	12828	1826	-	-	-
Gonyaulax spinifera	80	4088	623	666	8356	1221
G.polygramma	20	4500	807	-	-	-
G.verior	60	27040	4516	-	-	-
Prorocentrum micans	545	10996	1580	1000	16965	2376
P. minimum/ P. balticum	1400	9948	1439	4500	2535	397
P. gracile/P.sigmoides	160	15134	2134	250	13614	1932
P. arcuatum	233	13614	1932	986	14632	2067
P. donghaiense	3000	5320	799	6060	8181	1197
P. compressum	160	8181	1197	40	147262	18114
Pyrophacus steinii	140	61324	7950	840	56566	7369
Scrippseilla trochoidea	1153	11611	1419	1120	7942	1047
Protoceratium reticulatum	40	22437	3375	-	-	-
Heterotrophic						
D. rotundatum	80	9533	1382	-	-	-
Gyrodinium sp	14053	3743	574	48166	11240	1613
G. spirale	187	55127	7244	140	63689	10277
Gymnodinium catenatum	106	18750	3177	-	-	-
Oblea rotunda	100	70685	11359	-	-	-
Protoperidinium steinii	313	3983	608	780	5403	810
P. divergens	50	87967	14013	50		18283
P. depressum	40	220893	33916	-	-	-
P. curvipes	20	134628	21084	-	-	-
P cf. *minutum*	20	3515	636	-	-	-
P. subinerme	20	18840	3192	-	-	-
P. granii	20	5272	939	-	-	-
P. leonis	40	32500	5387	-	-	-
P. oblongum	-	-	-	40	149500	23316

Table 2 shows carbon biomass values in microgram per Liter in different cells size classes. Species with cell size between 20-45 µm contribute high carbon biomass ranging from 0.04 to 8.06 µg CL^{-1} at Stn A and 0.63 to 77.4 µg CL^{-1} at Stn B; cells with sizes 45-60 µm had low carbon biomass at Stn A (0.04 to 1.13 µg CL^{-1}) and high at Stn B (0.48 to 6.80 µg CL^{-1}); cells having 100-150 µm had carbon biomass ranged from 0.01 to 1.35 µg CL^{-1} at station A and 0.10 to 1.86 µg CL^{-1} at station B; Cell sizes between 200-450µm had carbon biomass ranging from 0.49 to 0.53 µg CL^{-1} at station A and 0.19 to 3.0 µg CL^{-1} at station B.

Discussion

This study provides new data on biovolume and carbon biomass of 45 marine dinoflagellate species from Pakistani coastal waters. Linear measurement of simple shapes, such as, ellipsoidal, spherical, double cone form, prolate ellipses, cones and half spheres were easily measured, but some species are more complex in shape, such as, "cone+3 cylinders", "ellipsoidal + 2 cones + cylinder", or "cylinder + cone" used for *Ceratium* sp. required more measurements[20]. A double cone shape has been recommended for the measurement of *C. fusus* and *C. inflatum* (Sun Jun, personal communication)..

In present study, biovolume and carbon content of dinoflagellates was recorded on single cell basis fall well within the range reported earlier[5,24,25,26]. Contribution of carbon biomass in a litter of water appeared to be controlled by the abundance of dinoflagellates species which vary with geographical location and other nutritional and environmental conditions. For example, heterotrophic species have been reported to have high carbon biomass (5 to 438 µg C L^{-1})[27], (0.5-272 µg CL^{-1})[25]. It is interesting to note that mixotrophs from the same area (North Sea) had lower range (0.2 to 455 µg CL^{-1})[25]. Carbon contribution in Pakistani coastal waters although lower but lie within the range reported in previous studies[25, 27]. As small dinoflagellates species are abundant in coastal waters of Pakistan, they contribute high amount of carbon compare to larger species regardless of nutritional modes, for example, both *P. minimum* (mixotroph) and *Gyrodinium* sp. (heterotroph) were observed in abundance having small volume but contribute more carbon (77 µg CL^{-1}) which is good agreement with the results of same size

Table 2. The total carbon biomass microgram per Liter values (µg CL^{-1}) of different size classes dinoflagellates estimated from Station A and Station B.

Size classes	Taxa	Station A	Station B
13-18µm	*Prorocentrum minimum/P.balticum*	0.86	1.79
20-45 µm	*Alexandrium ostenfeldii*	1.72	4.24
	A. tamarense	0.40	-
	Dinophysis rotundatum	0.16	-
	Gonyaulax spinifera	0.04	0.81
	G. polygramma	0.01	
	Gyrodinium sp	8.06	77.6
	Gymnodinium catenatum	0.33	-
	P. donghaiense	2.39	7.25
	P. compressum	0.19	1.45
	Protoperidinium steinii	0.19	0.63
	P. curvipes	0.42	-
	P cf. *minutum*	0.01	-
	P. subinerme	0.06	-
	P. granii	0.01	-
	Protoceratium reticulatum	0.13	-
	Scrippseilla trochoidea	1.63	1.17
45-60 µm	*A. tamiyaunichivi*	0.45	-
	Akashiwo sanguinea	0.04	-
	Cochlodinium sp	0.13	5.97
	D. fortii	0.34	-
	D. infundibula	0.04	-
	G. verior	0.27	-
	P. micans	0.86	2.38
	P. gracile/P.sigmoides	0.34	0.48
	P. arcuatum	0.45	2.03
	Pyrophacus steinii	1.11	6.18
	Oblea rotunda	1.13	-
75-150 µm	*Ceratium furca*	0.13	1.83
	C. kofoid	-	0.1
	C. lineatum	0.22	0.19
	C. longipes	0.01	
	C. macroceros var macroceros		0.15
	Dinophysis caudate	0.96	1.86
	D. miles	0.05	
	D. acuminata	0.37	
	Gyrodinium spirale		1.44
	Protoperidinium divergens	0.70	0.91
	P. depressum	1.35	
	P. oblongum	1.35	0.93
200-450 µm	*Ceratium fusus*	0.53	3.9
	C. inflantum	0.49	0.63
	C. massilliense	-	0.19

species (*Gyrodinium* sp) reported from Mediterranean waters[25]. Carbon contribution of large size dinoflagellates in Pakistani waters is much lower compared to other studies[6, 24, 25, 26, 28] primarily due to low cells densities of large size dinoflagellates species (Table 1).

The only species, *Gymnodinium* sp., has been studied for assessment of carbon biomass from eastern part of the Arabian Sea bordering India[22]. Dinoflagellates from Pakistani waters appear to have more cellular carbon (269-39116 pg C $Cell^{-1}$) as oppose to carbon levels reported by Ravi Kumar et al.[22]. Therefore, results obtained in the present study (269-39116 pg C $Cell^{-1}$ or 0.33-77.42 µg CL^{-1}) fall well within the range of carbon biomass reported from various waters (0.1 to 3.5 µg C L^{-1})[29] and (0.05 to 2.0 µg C L^{-1})[30]. Variation in carbon biomass is regulated by species composition, their cell sizes and nutritional conditions. For example, coastal species appear to have more carbon compared to open water species[27]. Similarly, thecate species have more carbon as oppose to athecate spesies[5]. In spite of their high biovolume, athecate species were less significant than the thecate species in terms of carbon contribution. Most of thecate species were small, but produce more carbon per unit volume per unit time[5].

In the present study Lugol's fixed samples were used for the estimation of biovolume and carbon biomass. However, Lugol's fixation is reported to causes shrinkage of cells, for example, in cyanobacteria[31], diatoms and dinoflagellates[32,33] and ciliates[34,35,36]. Although live samples have been used[33], but it is difficult to culture all species present in the natural waters.

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